APPROVED TECHNIQUE OF THE RIDEAL-WALKER TEST.

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The following details of the Rideal-Walker Test have been collated from the literature of the subject, published during the past ten years, and may be taken to represent the method as officially adopted at the present time in Great Britain and the British Colonies. In this, as in all other arbitrary tests, the need for strict observance of the conditions laid down by the authors cannot be too strongly emphasized. It has always been found when discrepancies in results obtained by the Rideal-Walker method were investigated, that one or more of the conditions called for in the test had been ignored. In the case of the Lancet Commission, for instance, the explanation was found to lie in the fact that the commissioners had been working with broth prepared from bullock's heart instead of Liebig broth, which had the effect of depressing all coefficients by about 50 per cent.

It is hoped, in presenting the following details of this test in a form which admits of easy reference, that the difficulties hitherto experienced by workers in this country in controlling the manufacture and sale of disinfectants may be met and overcome.

MATERIALS REQUIRED FOR TEST.

Nutrient Broth.

Liebig's Extract of Meat	20 grams
Peptone (Witte's)	20 "
Salt (Sodium Chloride, C. P.)	10 "
Distilled Water	1 litre

Boil the mixture for thirty minutes, then filter, and neutralize with normal sodium hydrate solution, using phenol-phthalein as indicator. In order to avoid contaminating the broth with phenol-phthalein it is advisable to take an aliquot part of the filtered broth—say 10 c. c.—and titrate this with decinormal sodium hydrate, calculating the amount of normal sodium hydrate necessary for the neutralization of the remainder of the broth. Add when quite neutral 15 c. c. of normal hydrochloric acid. This will give the broth a reaction of + 1.5 per cent. The broth is then made up to the litre, filtered and sterilized. Where two or three litres are prepared at one time, as is customary, the broth is distributed in 500 c. c. flasks on the following day and again sterilized. Five c. c. are then run with the aid of a small separating funnel into sterile test tubes,

which, after plugging with sterile cotton wool are placed in the steam sterilizer for half an hour or so.

Standard Carbolic Acid.

As carbolic acid crystals are very often contaminated by cresols to such an extent as to make them unreliable for purposes of bactericidal control, their purity should be established by a determination of the solidifying point (point of constant temperature) on at least 50 c. c. of the material with the thermometer in the liquid. The point is very sharp, the thermometer showing a constant temperature for a period of from five to ten minutes. The solidifying point of the crystals is 40.5 but anything over 40.0 may be accepted. A 5 per cent. (by weight) stock solution is then prepared and standardized by titration with decinormal bromine. From this solution (which keeps indefinitely in stoppered bottles) the various working strengths are made up by diluting some comparatively large quantity, such as 100 c. c., to the desired volume; this serves to eliminate the error introduced by measuring out small quantities of strong acid.

Dilutions of the Disinfectant.

A stock solution or emulsion should be prepared in 100 c. c. stoppered cylinder, with sterilized distilled water—10 per cent. if the coefficient be under 1, and 1 per cent. if over 1. Ten c. c. of this stock solution are used in preparing each of the 4 dilutions required for the test. Thus, working with a sample having a coefficient under 1, if it is desired to prepare a dilution of 1: 70, 10 c. c. of the 10 per cent. stock solution are diluted with 60 c. c. of distilled water, and in the case of a preparation having a coefficient over 1, where the dilution required is 1: 700, 10 c. c. of the 1 per cent. stock solution should be diluted with 60 c. c. of water. In preparing dilutions of the unknown, the limitations of the test must not be overlooked. The following is a safe rule for general work, expressing the dilutions as multiples of the carbolic acid dilution:—

With coefficients of 1 and under	x 0.1
With coefficients above 1 but not exceeding 10	x 0.5
With coefficients above 10 but not exceeding 20	x 1.0

For example, assuming that the strength of the carbolic acid control be 1: 100, when it is desired to test a sample having a coefficient of 10, the dilutions to be recommended would be 1: 950, 1: 1000, 1: 1050, 1: 1100.

The Broth Culture.

B. typhosus, grown in R. W. broth and incubated for 24 hours at 37° C. provides the test culture. To insure even distribution of the bacilli in the broth culture, and to avoid the necessity of filtration, the culture tube should be shaken and allowed to rest for half an hour before it is finally

removed from the incubator, the temperature of which should not vary more than half a degree from day to day. It is advisable to make a sub-culture every 24 hours from the previous 24-hour culture, even if on many days no test is to be performed; but as this tends to attenuate the organism it should be continued for not more than one month, after which a fresh sub-culture in broth should be taken from a month-old agar culture. By this means a culture not varying much from day to day in resistance to disinfectants is obtained, making the selection of the proper dilution

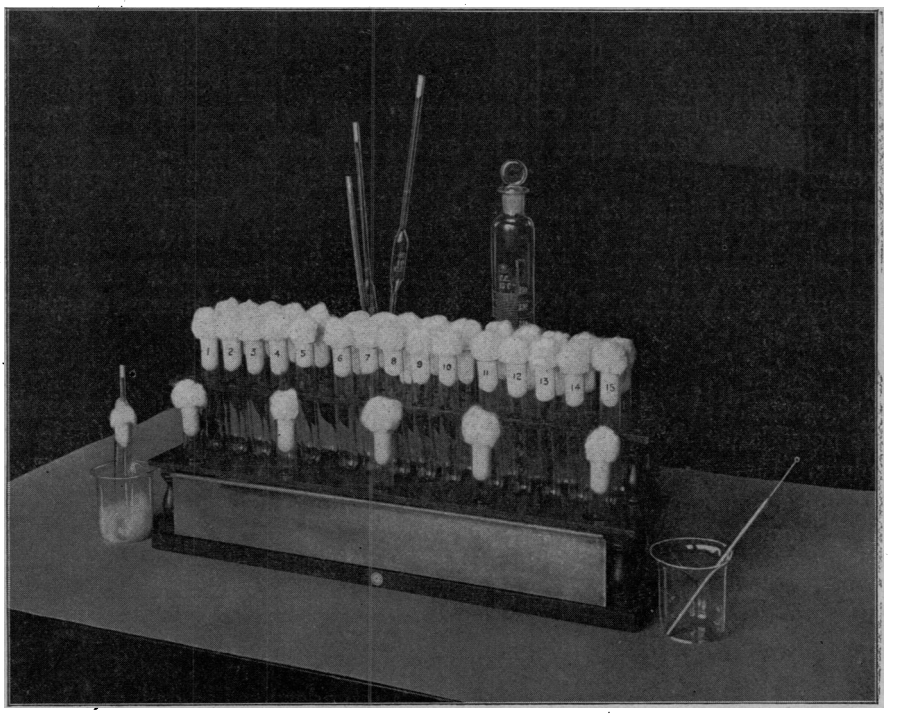

Fig. 1.

of carbolic acid much easier than it would be if the culture from which the 24-hour growth is obtained were older on one occasion than on another.

Apparatus Required for Test.

(The R-W apparatus as shown in Fig. 1 may be obtained from Arthur H. Thomas Company, of Philadelphia, Pa.)

Test Tube Rack.

A special rack is used. It contains two tiers, the upper having holes for 30 test tubes, in two rows, each row containing three sets of five; this tier is for the sterilized broth tubes, each of which is numbered with grease pencil. The lower tier is for the medication tubes—four with dis-

infectant dilutions and one with the carbolic acid control dilution, the latter being placed in the fifth hole. The lower tier is provided with a copper water bath to keep the temperature of medication within the prescribed limit—15° to 18° C. The test tubes being numbered in rotation it will be seen that the first medication tube is used for inoculating broth tubes, 1, 6, 11, 16, 21 and 26, the second for 2, 7, 12, 17, 22 and 27, etc.

Inoculating Needle.

The needle used should be composed of thin aluminium rod, with a short piece of platinum wire (26 U. S. gauge) passed through and twisted round an eye in the end of the rod, or otherwise firmly fixed thereto. The wire is made into a loop at the end and bent slightly in the center to allow of a fair sized drop being taken up for each inoculation. Satisfactory results cannot be expected when one tube is inoculated with a full drop and a mere film is introduced into another. The length of the wire to end of loop should be about $1\frac{3}{4}$ inches. After a little practice it is easy to obtain a satisfactory drop by dipping the needle in the medicated culture and bringing it out with a slight jerk. The loop used has an internal diameter of 3 m. m.

Test Tubes.

The test tubes should be of fairly strong glass so as to minimize as far as possible the risk of breakage, and lipped, to facilitate manipulation of plugs. Five inches by $\frac{5}{8}$ inch is the size recommended for use. The cotton wool plugs for both medication tubes and broth tubes should be well made, so that they can be withdrawn and replaced without loss of time. A convenient method is to place a thin flat piece of cotton wool over the mouth of the test tube, with a smaller piece in the center to form a core, and to push both into position with the aid of a thin glass rod.

Dropping Pipette.

This is used for the broth culture and is standardized to deliver 0.1 c. c. per drop. It is loosely plugged at the top with cotton wool, and when not in actual use is kept in a sterile test tube plugged at the mouth with cotton wool. For greater convenience the tube should be passed through the center of the plug and fastened thereto with wire.

In addition to the above, one or two each of the following are required: 1, 5 and 10 c. c. pipettes; 100 and 250 c. c. stoppered cylinders (with inverted breakers, to safeguard against dust after removal from sterilizer); wire baskets to receive tubes for incubation or sterilization. All pipettes and cylinders should be standardized.

Technique.

Before commencing the test it is necessary to ascertain the carbolic acid control dilution which will give the desired result — *i. e.*, life in $2\frac{1}{2}$

and 5 minutes. This is done by running a trial test with 5 dilutions of the carbolic acid only—say, 1: 80, 1: 90, 1: 100, 1: 110 and 1: 120. Five c. c. of the control solution so ascertained are then pipetted into the fifth medication tube, the other 4 receiving 5 c. c. of the various dilutions of the disinfectant under test. To save time and apparatus, one pipette can be made to do service at this stage by starting with the phenol solution and following on with the highest or lowest dilution of the disinfectant, according as the coefficient is below or above one, rinsing out the pipette in each case with the next dilution before measuring off the sample for test.

The plug of the culture tube is now replaced by the culture pipette which, as explained above, has a plug attached to it with wire, at such a height

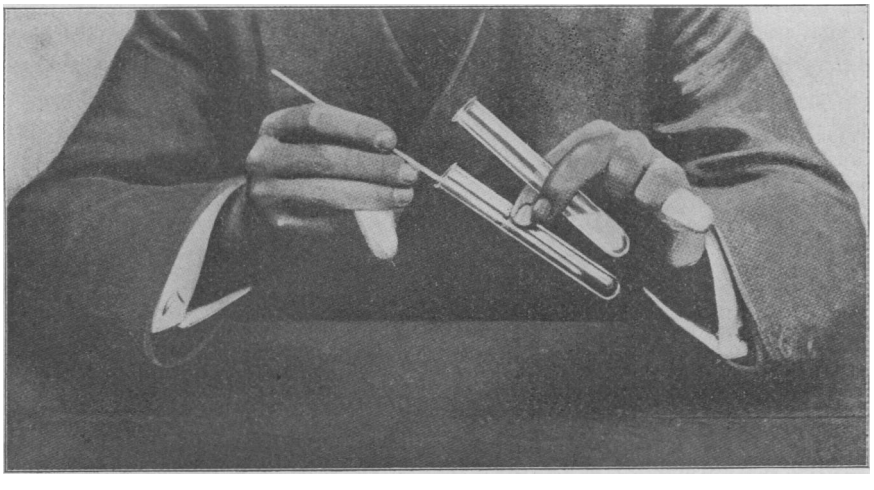

Fig. 2.

that when the plug fits easily into the mouth of the culture tube, the point of the pipette is half way down the broth, and clear of the clumps. The first of the five medication tubes is now inoculated with five drops of the culture—*i. e.*, 0.5 c. c. At intervals of half a minute each of the other medication tubes is inoculated in turn. By the time the fifth tube has been inoculated the organism in the first will have been exposed to the action of the disinfectant for two minutes, and after the next half minute a loopful of the latter is inoculated into the first broth tube, loopfuls from the other medication tubes being in turn inoculated into their respective broth tubes at the rate of one every thirty seconds, By the time the fifth broth tube has been inoculated from the fifth medication tube, the disinfectant in the first medication tube will have acted on the test organism for $4\frac{1}{2}$ minutes, and after the next thirty seconds a loopful is introduced

into broth tube 6, and so on. The actual test, therefore, occupies 17 minutes, and provides for six $2\frac{1}{2}$-minute periods of contact in each of the five medication tubes.

It is open to the worker, of course, to adopt any convenient method of manipulating the tubes and plugs. The following procedure is given for the guidance of the inexperienced. (See Fig. 2). The first medication tube is taken from the rack and the contents gently agitated for a second to ensure even distribution of the bacilli; the plug having been taken out and grasped by the left little finger, the tube is held between the back of the left forefinger and front of the second. The corresponding broth tube (No. 1) is taken up by the right hand and transferred to the left between the thumb and forefinger, the plug being extracted and held by the little finger of the right hand. The tubes now being in position for inoculation, the needle, which should have been sterilized before the tubes were touched, is introduced into the medication tube, from which a loopful is taken and inoculated into the broth tube. The needle is sterilized in the flame, (placed to the right) and pushed with a movement of the thumb well up between the first and second fingers of the right hand; the plugs are then replaced, the medication tube going back to the rack while the broth tube is subjected to a gentle agitation and placed in a wire basket on the right of the rack. This basket containing the thirty inoculation tubes and test form giving particulars of the dilutions, etc., is now placed in the incubator where it is allowed to remain for 48 hours at blood heat, when the results are read off. A moment's consideration of the manner in which the test has been conducted will suffice to indicate where the results of each subculture should be placed in the table.

The following details of a test of pyxol show the form in which the results are set out; incidentally it shows the degree of refinement to which the test can be carried with a little practice and care. (See page 581.)

The strength or efficiency of the disinfectant under test is expressed in multiples of carbolic acid, and is obtained by dividing the dilution of the disinfectant showing life in $2\frac{1}{2}$ and 5 minutes by the carbolic acid dilution, which, of course, must show the same result. In the present instance this 'figure of merit', or Rideal-Walker coefficient is 20.0.

To avoid annoyance and loss of time caused by aerial contamination of tubes, etc., it is advisable to conduct the test in a room free from draughts; a further safeguard is provided by spraying or swabbing the floors and benches with an efficient disinfectant solution. Needless to add, all pipettes etc., must be rigorously sterilized before use.

B. TYPHOSUS, 24 HOURS' BROTH CULTURE AT 37° C.

Temperature of Medication 15° to 18° C.

Sample.	Dilutions.	Time culture exposed to action of disinfectant—minutes.						Sub-Cultures.	
		2½	5	7½	10	12½	15	Period of Incub.	Temp.
Pyxol.....	1 : 1900	x	.....	.....	.....	.....	.....	48 hours	37° C
Pyxol.....	1 : 2000	x	x	.....	.....	.....	.....	"	"
Pyxol.....	1 : 2100	x	x	x	.....	.....	.....	"	"
Pyxol.....	1 : 2200	x	x	x	x	.....	.....	"	"
Carbolic Acid....	1 : 100	x	x	.....	.....	.....	.....	"	"

∴ Rideal-Walker Coefficient $\frac{2000}{100} = 20.0$.

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